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Introduction

The EZgene™ 96 Well Tissue RNA Kit provides an easy and fast method for isolating total RNA from animal tissues, including some difficult fibrous tissues such as skeletal muscle, heart and aorta tissue. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary. This kit purifies up to 50 μg of total RNA from animal tissues. The purified RNA is ready for RT-PCR.

Storage and stability

DNase I (optional) and Proteinase K should be stored at -20℃. All other components can be stored at room temperature. All kit components are guaranteed for 1 year from the date of purchasing.

Kit contents

Catalog#	R6811-00	R6811-01	R6811-02
Preps	1 x 96	4 x 96	20 x 96
96-well RNA Plate	1	4	20
96 Deep-well Plate	3	12	60
96-well Collection Plate (500 µL)	1	4	20
Buffer LY	40 mL	160 mL	2 x 400 mL
Buffer RB	125 mL	500 mL	5 x 500 mL
RNA Wash Buffer*	40 mL	2 x 80 mL	10 x 80 mL
DEPC-Treated ddH_2O	25 mL	100 mL	500 mL
Proteinase K	28 mg	4 x 28 mg	550 mg
DNase I (RNase-free, 2U/µL)**	500 U (250 µL)	2000 U (1000 µL)	5 x 2000 U (5.x.1mL)
1 x DNase I Buffer**	5 mL	20 mL	100 mL
DNase Stop Buffer	24 mL	100 mL	5 x 100 mL
RNase Inhibitor (40 U/µL) **	50 µL	200 µL	5 x 200 µL
User Manual	1	1	1

*DNase I and RNase Inhibitor not supplied. They could be purchased from Biomiga

*Add 160 mL (R6811-00) or 2 x 320 mL (R6811-01) or 10 x 320 mL (R6811-02) 96-100% ethanol to RNA Wash Buffer before use.

** DNase I and 1 x DNase I Buffer are shipped separately by ice.

Safety information

Buffer LY contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

Before starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- ☑ Determine the volume of Buffer LY to be used and add 20 µL of β-mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains β-ME can be stored at room temperature for up to 1 month.
- ☑ Crystals may form in Buffer LY, dissolve the precipitates at 37°C before use.
- ☑ Add 160 mL (R6811-00) or 2 x 320 mL (R6811-01) or 10 x 320 mL (R6811-02) 96-100% ethanol to RNA Wash Buffer before use.
- ☑ Reconstitute Proteinase K with 1.1 mL (R6811-00) or 4 x 1.1 mL (R6811-01) or 22 mL (R6811-02) DEPC-Treated ddH_2O, Vortex vial briefly and aliquot into adequate portions. Store aliquots at -20 °C.
- ☑ Add 36 mL (R6811-00) or 150 mL (R6811-01) or 5 x 150 mL (R6811-02) 100% ethanol to DNase Stop Buffer before use.
- ☑ All centrifuge steps must be carried out at 22 °C.

Materials supplied by users

- ☑ Centrifuge with swinging-bucket capable of 4000 g
- ☑ Rotor and adapter for 96-well plate
- ☑ Water bath or heat block preset at 55 °C
- ☑ 100% ethanol
- ☑ β-mercaptoethanol
- ☑ RNase-free pipette tips

Disruption and homogenization of tissue samples

It is critical to disrupt and homogenize the samples completely and properly for high quality RNA yield. The purpose for homogenization is to reduce the viscosity by shearing genomic DNA and other high molecular weight cell components to create a homogenous lysate. Incomplete homogenization may result in clogging the well and reducing the RNA yield.

1. **Sample disruption by mortar and pestle**

- Excise tissues and freeze in liquid nitrogen immediate.
- Grind the sample with ceramic mortar and pestle to a fine powder under liquid nitrogen.
- Transfer the suspension into a tube pre-chilled in liquid nitrogen and allow the liquid nitrogen to evaporate while the samples remain frozen.
- Add Buffer LY before the sample gets thawed.

2. **Homogenization using homogenization columns**

It is a fast and efficient way to homogenize the samples using **Biomiga's homogenization column.** Up to 700 µL of samples can be loaded per column. Homogenization columns are supplied in the Plant RNA Kit and can be purchased separately for use with the tissue RNA kit.

3. **Rotor-Stator for sample disruption and homogenization**

Using a proper size probes and generator, the process simultaneously disrupts and homogenizes most of samples.

4. **Bead milling for sample disruption and homogenization**

Cells and tissues can be disrupted and homogenized by rapid agitation in the presence of **glass beads** in Buffer LY. Use 4-8 mm glass beads for animal tissues, 0.5 mm for yeast cells and 0.1 mm for bacterial samples.

Removal of genomic DNA using DNase digestion

DNA digestion is necessary for downstream applications that are sensitive to very small amounts of DNA, for example, RT-PCR with low-abundance target. Generally, it is not required to do so since the EZgene RNA purification kit selectively isolates RNA and eliminates most of the DNA. If there is DNA contamination, either reduces the tissue amount or cell

Stabilization of RNA in harvested animal tissues

The intact of RNA in harvested tissue will be protected with the addition of **RNASecure solution (Biomiga, catalog# R1011)**.

1. Cut the tissue into slices less than 0.5cm thick and immediately add at least 15 volumes of RNAsecure solution, for example, 150 uL RNAsecure solution per 10 mg tissue.

2. Store at room temperature for up to 24 hours, at 4℃ for up to a week, and –20 ℃ or –70 ℃ for long term.

RNA quality

It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to each well. An A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

EZgene™ 96-Well Tissue RNA Protocol

1. Excise the tissue sample from animal or from storage.

2. Weight **10 mg** tissue and place it into suitable vessel for disruption and homogenization. ***Do not use more than 15 mg tissue.***

3. Add **300 µL Buffer LY/β-mercaptoethanol (β-ME)** and disrupt tissue and homogenize issue in Buffer LY as described in previous section.

   Note: Rotor-Stator or Beads Mills normally can result in higher RNA yield because they provided better disruption and homogenization.

   Note: β-mercaptoethanol is key in denaturing RNases and must be added to an aliquot of Buffer LY before use. Add 20 µL of β-mercaptoethanol per 1 mL of Buffer LY. This mixture can be stored for 1 week at room temperature.

4. Transfer the homogenized lysate into a **new 96 Deep-Well plate** (Supplied).

5. Pipet **600 µL DEPC-Treated ddH_2O** to each sample and add **10 µL proteinase K** solution and mix throughly by pipetting. Incubate at 55°C for 10 minutes.

6. Centrifuge at 4,000 x g for 15 minutes at room temperature. A thin layer or film will form on top of the supernatant.

7. Transfer the supernatant into a **new 96 Deep-Well plate**. (Supplied).

Note: after transfer the supernatant, keep the 96 Deep-well plate for the use of collection for step 9.

Note: Avoid transferring any of pellet. Hold the pipett tip under the thin layer or film on top of the supernatant, if present. This layer will usually adhere to outside the tip and should not be transferred.

8. Add **0.5 volume** (around 450 µL) of **absolute ethanol** (96-100%) to the cleared lysate, mix throughly by pipetting or vortexing.

9. Place an **EZgene™ 96 Well RNA Plate** on top of the **96 Deep-well plate** from step 4.

10. Carefully apply **700 µL** samples from step 8 (including any precipitate) to **EZgene™ 96 Well RNA Plate.**

11. Spin at 4000 x g for 5 min at room temperature.

12. Transfer the remaining samples to **EZgene™ 96 Well RNA Plate**.

    Note: after transfer of the lysate, keep the **96 Deep-well plate** for the use of collection for step 14.

13. Centrifuge at 4000 x g for 5 minutes.

14. Remove the **EZgene™ 96 Well RNA Plate** and place it onto a **96 Deep-Well plate** from step 12.

15. Pipet **350 µL Buffer RB** into each well. Centrifuge at 4,000 x g for 5 minutes.

16. **Prepare the DNase I solution:** For 96 samples, in a 15 mL microtube, add **250 µL DNase I** and **100 µLRNase Inhibitor** to **7.15 mL 1 x DNase I Buffer** Mix gently by inverting the tube. Do not vortex, DNase I is especially sensitive with physical denaturation.

17. Add **75 µL DNase I solution** onto the middle of each well of **EZgene™ 96 Well RNA Plate** and incubate at room temperature for 15 min. Add **200 µL DNase Stop Buffer** onto each well and centrifuge at 4,000 x g for 5 min.

18. Add **200 µL RNA Wash Buffer** to each well and centrifuge at 4,000 x g for 5 min.

19. Add **800 µL Buffer RB** to each well of the **EZgene™ 96 Well RNA Plate**. Wait 5 minutes and Centrifuge at 4000 x g for 5 minutes at room temperature.

20. Place the **EZgene™ 96 Well RNA Plate** on to of a **new 96 Deep-Well Plate**. Add **800 µL RNA Wash Buffer** to each well of the **EZgene™ 96 RNA Plate**. Centrifuge at 4000 x g for 5 minutes at room temperature.

21. Add another **800 µL RNA Wash Buffer** to each well of the **EZgene™ 96 Well RNA Plate**. Centrifuge at 4000 x g for 10 minutes at room temperature.

22. Place the **EZgene™ 96 Well RNA Plate** on top of a new **96 well collection plate** (supplied).

23. Add **75 µL DEPC-Treated ddH_2O** onto center of membrane in each well of the plate. Centrifuge at 4000 x g for 4 minutes at room temperature.

24. Repeat the elution step (step 23) once with second **75 µL DEPC Treated ddH_2O**.Seal the plate contains purified RNA and store at -70℃

Troubleshooting Guides

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the membrane	● Repeat elution ● Pre-heat DEPC-water to 70℃prior to elution ● Incubate for 5 min with water prior to elution
	Overloaded sample	Reduce quantity of starting material
Clogged wells	Incomplete lysis	● Mix thoroughly after addition of Buffer LY ● Reduce amount of starting material
Degraded RNA	Source	● Do not freeze and thaw sample more than once ● Follow protocol closely, and work quickly ● Low concentration of virus in the sample
	RNase contamination	● Ensure not to introduce RNase during the procedure ● Check buffers for RNase contamination
Problem in downstream applications	Salt carry-over during elution	● Ensure RNA Wash Buffer has been diluted with 4 volumes of 100% ethanol as indicated on bottle ● 1 X RNA Wash Buffer must be stored at room temperature ● Repeat wash with RNA Wash Buffer
	Inhibitors of PCR	● Use less starting material ● Prolong incubation with Buffer LY to completely lyse cells
DNA contamination		Make sure to perform RNase-free DNase Digestion correctly
Abnormal OD reading on A260/A280	DEPC residue remains in DEPC-water	● Remove DEPC by Autoclave ● Use 10mM Tris-HCl, not the DEPC water to dilute the sample before measuring purity

Related EZgene™ products

Catalog #	Product Name	Preps	Price $
R6311-01	Tissue RNA kit	50	150.00
R6311-02	Tissue RNA kit	250	650.00
R1011-01	RNASecure Solution	50 mL	45.00
R1011-02	RNASecure Solution	250 mL	150.00
R6312-01	Tissue RNA midi kit	10	80.00
R6312-02	Tissue RNA midi kit	20	160.00
R6314-01	Tissue RNA maxi kit	10	120.00
R6314-02	Tissue RNA maxi kit	25	270.00
R6811-01	96-well tissue RNA kit	4x96	780.00
R6811-02	96-well tissue RNA kit	20x96	3300.00
R6411-01	Blood RNA mini kit	50	150.00
R6411-02	Blood RNA mini kit	250	680.00
R6812-01	96-well blood RNA kit	4x96	780.00
R6812-02	96-well blood RNA kit	20x96	3500.00

Limited use and warranty

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.